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Nanoparticles Induced Oxidative Stress: A Review Based Approach

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Abstract: *Nanotechnology, a rapidly evolving science, has generated game-changing breakthroughs in the industrial, medical, and consumer industries. Engineered nanoparticles (NP) are extremely attractive in a range of applications due to their unique physicochemical and electrical characteristics. Changes in NP's structural and physicochemical properties can alter their biologically active compounds, along with the generation of free radicals (ROS), one of the most widely documented Nano particle-related toxins. Cellular parameters such as surface of the particle, size, contents of composition, and metal content produce oxidative stress, whereas cellular responses such as mitochondrial functions, Nanomaterials cell contact, and immune cell activation cause ROS-mediated destruction. It's crucial to understand how NP influences the ROS response although the oxidative stress is a key predictor of Nanoparticle induced damage. It may be possible to develop a comprehensive toxicity screen that uses oxidative stress as a prediction model for nanoparticle damage, as well as a better understanding of the multiple signalling cascades activated by nanomaterials ROS..*

Key Words: Engineered Nanoparticles, Oxidative Stress, Physicochemical Characteristics, Reactive Oxygen Species (ROS)

Introduction

The extensive use of nanoparticles raises worries about unintended human exposure and the resulting negative health impacts (Donaldson, Murphy, Duffin, & Poland, 2010). (Shvedova, Pietroiusti, Fadeel, & Kagan, 2012). Only a little amount of study has been done to investigate the possible harmful consequences of these modified NM, in comparison to the expanding economic interest in NM. The vast range of physicochemical features of NM, including structural and elemental composition, makes assessing their dangerous consequences complicated and time-consuming (Ju-Nam & Lead, 2008; Sattler, 2010) (Sattler, 2010). The majority of research to date has revealed that NP toxicity is commonly associated with ROS production (which will be beneficial or detrimental during its biological interactions) and oxidative stress (Nel, Xia, Madler, & Li, 2006). Because several of NP intrinsic features can accelerate the generation of ROS, physicochemical characterization of nanoparticles, including particle size, its surface charge density, and its chemical composition, is a critical indication for the resultant ROS response and

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Nanoparticle produced damage (Shvedova et al., 2012).

CNT-induced oxidative stress activates cell signaling pathways giving rise in the production of proinflammatory and fibrotic cytokines NP-mediated ROS responses had been related to a multitude of negative effects, including genotoxicity, fibrosis, inflammation, and carcinogenesis (Li, Muralikrishnan, Ng, Yung, & Bay, 2010). Some NP have been proven to stimulate inflammatory reactions including neutrophils and macrophages, resulting in a rise in the formation of reactive oxygen species (Zhang, Berg, Levanon, Fessenden, & Meisel, 2003) (Kennedy, Wilson, & Barakat, 2009). The process for ROS synthesis differs for each nanoparticle, and the actual underlying cellular mechanisms and for ROS creation is yet unknown (Fubini & Hubbard, 2003) (Y.-W. Huang, Wu, & Aronstam, 2010). However, ROS formation is not necessary for NP-induced toxicity, as a different publications had documented nanoparticle toxicity without producing ROS (He et al., 2011).

Oxidative Stress

An mismatch among reactive species, often known as free radicals, and antioxidants (Finaud, Lac, & Filaire, 2006).

- Cells can balance the synthesis of oxidants and antioxidants under normal circumstances.
- Free radicals are reactive chemicals that the human body naturally produces.
- They can have both beneficial and harmful effects (for example, on the immunological system) (e.g. lipids, proteins or DNA oxidation).
- Physical activity also causes changes in homeostasis by increasing oxidative stress.
- Furthermore, oxidative stress appears to have a role in muscle exhaustion, which might contribute to overtraining.
- When our cells are out of equilibrium owing to an increase in amount of free radicals or a reduction in antioxidants, we call it oxidative stress.
- Oxidative stress disrupts the development of cellular structures by causing structural alterations in the cell wall. It also resulted in a change in function and, finally, death.

Most Common Causes of Oxidative Stress (Finaud et al., 2006)

Endogenous Sources

Immune cell activation, Inflammation, Mental stress, Body's natural utilization of oxygen as breathing and some cell-mediated immune processes

Exogenous Sources

Exogenous sources like pollutants in the environment, cigarette smoking and radiations

Oxidative Stress Effects in the Body

An excess of reactive oxygen species (ROS) can cause potentially harmful cellular reactions, culminating in the oxidative stress syndrome. It's caused by a mismatch between ROS generation and a biological system's ability to quickly detoxify reactive intermediates or repair the harm . Cells can engage enzymatic and nonenzymatic antioxidant mechanisms to counteract the excessive ROS response (Sies, 1991). To illustrate a mechanism for NP-mediated oxidative stress, the hierarchical model of oxidative stress was presented (Y.-W. Huang et al., 2010). According to this hypothesis, when cells and tissues are exposed to NP, antioxidant enzyme systems respond to increased levels of oxidative stress. Nuclear factor (erythroidderived 2)-like 2 (Nrf2) stimulation causes transcriptional activation of phase II antioxidant enzymes after moderate oxidative stress. A proinflammatory response is mounted at an intermediate level via the redox-sensitive mitogen-activated protein kinase (MAPK) and nuclear factor kappa-light-chain enhancer of activated Bcells (NF-B) cascades. Extremely high levels of oxidative stress, on the other hand, cause mitochondrial membrane breakdown and electron chain malfunction, which leads to cell death. Extremely high levels of

oxidative stress, on the other hand, cause mitochondrial membrane breakdown and electron chain malfunction, which leads to cell death. The depletion of antioxidants or the enhanced generation of reactive oxygen species (ROS) are two main mechanisms that promote the prooxidant effects of modified NM. Peroxide and free radical generation is increased when the usual redox state is disrupted, which has negative consequences for cell components such as proteins, lipids, and DNA (C.-C. Huang, Aronstam, Chen, & Huang, 2010). Because of its chemical reactivity, oxidative stress may cause DNA damage, lipid peroxidation, and signalling network activation, all of which are linked to cell death, fibrosis, and carcinogenesis (Knaapen, Borm, Albrecht, & Schins, 2004). Apart from cellular damage, ROS can be produced by NP interactions with a variety of biological targets as a result of cell respiration, metabolism, ischemia/reperfusion, inflammation, and NM metabolism. Airway inflammation and interstitial fibrosis are caused by oxidative stressors caused by occupational NM exposures as well as experimental challenges with different NP (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006).

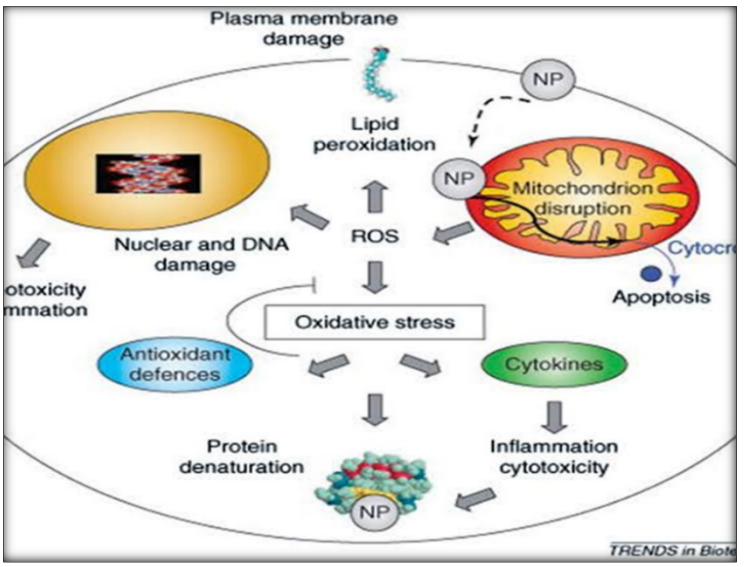

Figure 1: Cellular Responses to Oxidative Stress Generated by Nanoparticles

Generation of ROS

Within the cell, ROS are produced either internally or extrinsically (Risom, Møller, & Loft, 2005). ROS are reactive oxygen species, which are important signalling molecules in cell signalling and homeostasis. Superoxide anion (O_2), hydroxyl radical (OH), hydrogen peroxide (H_2O_2), singlet oxygen (1 O_2), and hypochlorous acid (HOCl) are only few of the oxidative species that make up ROS. Through one-electron reduction mediated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, molecular oxygen produces O_2, the principal ROS. By dismutation and metal-catalyzed Fenton reaction, further oxygen reduction might result in H_2O_2 or OH (Vallyathan & Shi, 1997)

Exogenous ROS inducers include artificial NM and environmental toxins, where endogenous ROS sources include mitochondrial respiration, inflammatory response, microsomes, and peroxisomes. ROS are created in trace levels in the body in response to numerous stimuli. Free radicals are produced as byproducts of mitochondrial respiration and Fenton-type processes mediated by transition metal ions (Thannickal & Fanburg, 2000). As a defensive

strategy against environmental contaminants, tumour cells, and microorganisms, inflammatory phagocytes such as neutrophils and macrophages cause oxidative outburst (Risom et al., 2005). One of the main mechanisms of cytotoxicity is the production of reactive oxygen species (ROS) by a variety of NP, including metal oxide particles. The formation of free radicals by NP has been shown to affect intracellular calcium concentrations, activate transcription factors, and modify cytokine production (C.-C. Huang et al., 2010).

Nanoparticle-Induced Oxidative Stress

Various nanomaterials, including as fullerenes,metal oxides and carbon nanotubes, have been found to cause oxidative stress (Vallyathan & Shi, 1997).The early event that leads to NP-induced damage is the production of reactive oxygen species (ROS) and oxidative stress.The physicochemical reactivity of NP, including metal-based particles and fibrous CNT, correlates to oxidative stress .

The important factors in NP-induce oxidative stress are: (Bonner, 2007)

- On the reactive surface of NP, there are prooxidant functional groups.
- Transition metal-based NPs cause active redox cycling on the surface of NPs.
- Interactions between particles and cells

Oxidative Stress is the Fundamental Mechanism of NP Toxicity

Free radicals generated when singular radical binds to the the surface of nanoparticles. On quartz particles, surface bound radicals such as [SiO] and SiO_2 are responsible for the generation of ROS such as OH and O_2 (Fubini & Hubbard, 2003). Because of their surface characteristics, Nanoparticle i.e. Si and Zn having same particle size or shape produces multiple cytotoxicity responses. Because ZnO is more chemically active than SiO2, it produces more oxygen, resulting into oxidative stress .Free radicals can be produced as free entities into aqueous solution or directly bound to the NP surface. The release of metal ions during NP dissolution can boost the ROS response (Knaapen et al., 2004) (Fubini & Hubbard, 2003). Oxidative stress caused by NP exposure includes mitochondrial function, necrosis, activation of the NADPH oxidase system, disruption of calcium homeostasis, and deficiency of antioxidant enzymes. The stimulation of cell signalling pathways, chemokine and inflammatory cytokine production, and particular transcription factor activation are all aided by the NP-driven ROS response. The activation of these cellular pathways is linked to the transcription of genes implicated in inflammation, genotoxicity, fibrosis, and cancer, suggesting that the side effects of NP exposure may be due to ROS production (Sioutas, Delfino, & Singh, 2005; Xia et al., 2006).

Particle-Cell Interactions as a Source of Oxidants

In contrast to self-oxidative nature, NP engages to the cells and generates pro-oxidant effects via internalized ROS formation that includes mitochondrial function and intiation of NADPH-like enzyme reactions (Driscoll et al., 2001). The cellular redox mechanism in the lungs, where leukocytes such as phagocytic cells and neutrophils act as direct ROS stimulants, can be activated by nanoparticles (Fadeel & Kagan, 2003).

Some of the NP physicochemical features are responsible for the phagocytic oxidative outburst. ROS itslef induced inflammation was linked to the particles' surface-based radical producing characteristics in the case of quartz and silica particles. Furthermore, chemical adsorption onto the NP surface, such as organic debris, may contribute to inflammation-induced oxidative stress (Sies, 1991).

Apoptosis within Metal Nanoparticles and Mechanism of ROS

The induced oxidative cell death caused by NP has been related to apoptosis as a crucial mechanism. Since mitochondria are among the primary targets for NP-induced peroxidation, the intrinsic

pathway apoptosis cascade plays an important role in metallic NP-induced apoptosis. (Hsin et al., 2008).

Elevated concentrations of ROS in the mitochondrial may destroy lipid membranes, resulting in depression of the mitochondrial matrix. A tiny amount of electrons leave the mitochondrial chain and unite with molecular oxygen to create O_2, which is then converted to H_2O_2 or partially decreased to the toxic $[OH]^-$ (Xia et al., 2006). By inhibiting its electron transport chain or speeding electron transfer into molecular oxygen, NP can accelerate O_2 production (Lenaz, 2001).

Carbon Nanotubes

CNTs are one of advanced materials along with their vast range of uses make its unique physical, chemical, and electrical properties.The structure of CNTs makes it easier for them to enter, deposit, and stay in the lungs and pleura, resulting in partial phagocytosis and clearance from the lungs (Stellaa, 2011). CNT are thought to have physiologically hazardous consequences because to their biopersistent and nonbiodegradable nature, as well as their similarity to needle-like asbestos fibres (Lam, James, McCluskey, & Hunter, 2004).The prooxidant effects of CNT are caused by physicochemical factors such as particle size, surface modification, metal presence, surface reactivity, and surface charge. CNT-induced oxidative stress has been linked to frustrated CNT phagocytosis(Stellaa, 2011).

Oxidative Stress Caused by Carbon Nanotubes

Among the most commonly reported toxicity outcomes for CNT is the creation of oxidative stress (ROS), which can be beneficial or detrimental during biochemical processes. The effects of internalised CNT on aerobic metabolism or the reduction of antioxidant components within the cell may lead to oxidative stress directly or indirectly through CNT-induced active oxygen species materials into the environment and inside the cell (Park, Choi, Park, & Park, 2008). Mitochondrial failure is the most plausible mechanism for CNT-induced oxidative stress and pulmonary damage (Shvedova et al., 2008). The presence of transition metals and particular reactive groups on the CNT surface, as well as incomplete phagocytosis of CNT, are all important causes of ROS formation. Metal impurities added into CNTs during synthesis, such as Fe, Co, and Ni, are important drivers of CNT-mediated ROS response (Le Goff, Holzinger, & Cosnier, 2011) (Warheit et al., 2004).

Approaches to Access Oxidative Stress in Clinical Sample

Measurement of Reactive Oxygen Species Directly

The primary molecules responsible for the negative consequences of oxidative stress are reactive oxygen species (ROS). One method of determining oxidative stress situations is to measure their cellular levels directly. The use of fluorogenic probes is one approach to determine the cellular levels of ROS (Schieber & Chandel, 2014) (Ubezio & Civoli, 1994). Superoxide molecules (O2), on the other hand, may be identified after staining with dihydroethidium, a fluorescent probe (DHE). Ethidium bromide decreased with sodium borohydride is similarly permeable to living cells. DHE is immediately converted to ethidium bromide by the superoxide anion inside the cells, which then fluoresces. The red fluorescence, which is measured at 488 nm and 585 nm, is thus thought to be proportional to the intracellular anion levels of superoxide (Benov, Sztejnberg, & Fridovich, 1998). The derivatives of all reactive oxygen metabolites (D-Roms) assay, as published by Trotti et al., is another approach to measure ROS compounds such as hydroperoxides (R-OOH), notably in blood (Trotti, Carratelli, & Barbieri, 2002) (Marnett, 1987).

Oxidative Stress Markers

A biomarker, often known as a marker, is a chemical substance or cellular event that serves as a biological status indicator. Markers are used to determine the amount of reactive oxygen species

in the body.

Oxidized DNA Damage Markers

The hydroxylation of deoxyguanosine residues produces 8-hydroxy-2′-deoxyguanosine (8-OHdG), is one of the most common oxidative changes into DNA. Enzymatic repair mechanisms may remove 8-OHdG residues from DNA, allowing them to circulate in the bloodstream and be excreted in the urine. 8-OHdG levels in patients' blood and/or urine can thus be used as a marker for oxidative DNA damage. 8-OHdG may be identified and quantified in urine samples using HPLC and GC-MS techniques (Akçay, Saygılı, Andican, & Yalçın, 2003).

Oxidized Protein Damage Marker

Using Western Blots Kits to detect selectively oxidized protein. 2, 4-Dinitrophenlyhydrazine (DNPH) kits are the most popular means of identifying oxidized proteins. The DNPH kit uses a western blot to identify DNPH carbonyl conjugates that are generated by attaching to an anti-DNP antibody (Levine et al., 1990).

The DNPH kit uses a western blot to identify DNPH carbonyl conjugates that are generated by attaching to an anti-DNP antibody (Mesquita et al., 2014).

Lipid Peroxidation Markers

Lipid peroxidation has long been employed as a marker for ROS-induced cell membrane damage (Halliwell & Gutteridge, 1986). The oxidative breakdown of lipid membranes is known as lipid peroxidation. It's a three-step chain reaction in which free radicals steal electrons from lipid cell membranes, causing cell death and oxidative stress. Lipid peroxides are unstable and breakdown into reactive by-products when exposed to air. Lipid peroxidation markers are used to identify a frequent lipid peroxidation by-product. These tests check for malondialdehyde (MDA),4-hydroxynonenal (4-HNE), and acrolein, among other compounds (Marshall, Warso, & Lands, 1985).

A New Biomarker Caused by Neutropihils

Hypochlorous acid (HOCL) and hypobromus acid are formed by myeloperoxidase (found in neutrophils) and eosinophil peroxidase (found in eosinophils). These reactive metabolites are necessary for the synthesis of 3, dibromotyrosine, which may be employed in oxidative stress immunohistochemistry (C.-C. Huang et al., 2010).

Antioxidant Assay

The antioxidant system in the body helps to counteract the detrimental effects of oxidative free - radical. When the ratio between antioxidants and ROS radicals, known as redox homeostasis, is disrupted, oxidative stress emerges. This prooxidant and antioxidant equilibrium can be altered by enhanced free radical generation, antioxidative enzymes inhibition, or high antioxidant consumption. As a result, clinical samples' concentrations of oxidative stress can be related to antioxidant status (Halliwell & Gutteridge, 1986).

We test the antioxidant's activity by comparing its absorbance to a known quantity of uric acid. Put uric acid in a well, dilute it, add antioxidants, copper, and a chromogen, and monitor activity (by measuring absorbance of the well) (McCord & Fridovich, 1969).

Assay used to Measure Reactive Oxygen Species (Mesquita et al., 2014)

Techniques	Mechanism
Fluorescent dye 2,7-dichloro fluorescin (DCFH)	The dye is produced as a diacetate precursor in this assay, which is cleaved by high pH to produce the non-"fluorescent product DCFH .The presence of ROS changes DCFH to a fluorescent product, 2,7dichloro"uorescein, which may also be detected by "Fluorimetry."
Plasmid assay	This test involves the unwinding and interpolation of a twisted bacterial DNA plasmid to detect free radical and/or ROS consumption.

Techniques	Mechanism
DCFH assay	ROS creation by carbon-black nanoparticles was amplified in the case of metal salts such as Ferric chloride, $FeSO_4$, and Copper sulfate, according to the DCFH test, showing that nanomaterials and metal ions interacted to promote Production of ROS.

Conclusion

This article addresses the pathways involved of NP-induced osmotic damage.With regard to the oxidative stress paradigm, we focus on the toxicity of metal oxide NP and CNT. The main causes of NP-induced oxidative stress are (a) the oxidative characteristics of the NP and (b) the formation of oxidants when the NP interacts with cellular material. The physicochemical features of NP, such as surface reactivity, particle size, surface charge, chemical composition, and the presence of transition metals, are responsible for their direct prooxidant effects. As a result, comprehensive physicochemical property characterization is required to assure the safe design and fabrication of NP. ROS caused by NPcell contact, on the other hand, is mediated by immune cell activation, mitochondrial respiration, and the NADPH oxidase system, among other processes. Cell membrane destruction, lipid peroxidation, protein denaturation, and changes in calcium homeostasis are the main pathophysiological effects of oxidative assaults caused by metal NPs.Furthermore, the results of the review paper imply that CNT-induced oxidative stress is a sign of CNT's pulmonary toxicity. The activation of these signalling pathways over time has clinical implications. Engineered.NP causes redox imbalance, which leads to pathophysiological effects such genotoxicity, inflammation, fibrosis, and carcinogenesis.Understanding the molecular and cellular processes of NP-induced oxidative stress is critical because it will lead to creative techniques for reducing the toxicity of modified NP.It also involves the implementation of strict processes for assessing the oxidative potential of synthesised NP before to commercialization. Identifying the key cellular targets for NP-induced ROS would help to make NM safer to develop and produce in the market.

References

Akçay, T., Saygılı, I., Andican, G., & Yalçın, V. (2003). Increased formation of 8-hydroxy-2 -deoxyguanosine in peripheral blood leukocytes in bladder cancer. *Urologia internationalis, 71*(3), 271-274.

Benov, L., Sztejnberg, L., & Fridovich, I. (1998). Critical evaluation of the use of hydroethidine as a measure of superoxide anion radical. *Free radical Biology and medicine, 25*(7), 826-831.

Bonner, J. C. (2007). Lung fibrotic responses to particle exposure. *Toxicologic pathology, 35*(1), 148-153.

Donaldson, K., Murphy, F. A., Duffin, R., & Poland, C. A. (2010). Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. *Particle and fibre toxicology, 7*(1), 1-17.

Driscoll, K. E., Howard, B. W., Carter, J. M., Janssen, Y. M., Mossman, B. T., & Isfort, R. J. (2001). Mitochondrial-derived oxidants and quartz activation of chemokine gene expression Biological *Reactive Intermediates VI*, 489-496

Fadeel, B., & Kagan, V. E. (2003). Apoptosis and macrophage clearance of neutrophils: regulation by reactive oxygen species. *Redox Report, 8*(3), 143-150.

Finaud, J., Lac, G., & Filaire, E. (2006). Oxidative Stress. *Sports Medicine, 36*(4), 327-358. doi: 10.2165/00007256-200636040-00004

Fubini, B., & Hubbard, A. (2003). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis. *Free radical Biology and medicine, 34*(12), 1507-1516.

Halliwell, B., & Gutteridge, J. M. (1986). Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. *Archives of biochemistry and biophysics, 246*(2), 501-514.

He, X., Young, S.-H., Schwegler-Berry, D., Chisholm, W. P., Fernback, J. E., & Ma, Q. (2011). Multiwalled carbon nanotubes induce a fibrogenic response by stimulating reactive oxygen species production, activating NF-κB signaling, and promoting fibroblast-to-myofibroblast transformation. *Chemical research in toxicology, 24*(12), 2237-2248.

Hsin, Y.-H., Chen, C.-F., Huang, S., Shih, T.-S., Lai, P.-S., & Chueh, P. J. (2008). The apoptotic effect of nanosilver is mediated by a ROS-and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. *Toxicology letters, 179*(3), 130-139.

Huang, C.-C., Aronstam, R. S., Chen, D.-R., & Huang, Y.-W. (2010). Oxidative stress, calcium homeostasis, and altered gene expression in human lung epithelial cells exposed to ZnO nanoparticles. *Toxicology in vitro, 24*(1), 45-55.

Huang, Y.-W., Wu, C.-h., & Aronstam, R. S. (2010). Toxicity of transition metal oxide nanoparticles: recent insights from in vitro studies. *Materials, 3*(10), 4842-4859.

Ju-Nam, Y., & Lead, J. R. (2008). Manufactured nanoparticles: an overview of their chemistry, interactions and potential environmental implications. *Science of the total environment, 400*(1-3), 396-414.

Kennedy, I. M., Wilson, D., & Barakat, A. I. (2009). Uptake and inflammatory effects of nanoparticles in a human vascular endothelial cell line. *Research Report (Health Effects Institute) 136*, 3-32.

Knaapen, A. M., Borm, P. J., Albrecht, C., & Schins, R. P. (2004). Inhaled particles and lung cancer. Part A: Mechanisms. *International Journal of Cancer, 109*(6), 799-809.

Lam, C.-W., James, J. T., McCluskey, R., & Hunter, R. L. (2004). Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicological sciences, 77*(1), 126-134.

Le Goff, A., Holzinger, M., & Cosnier, S. (2011). Enzymatic biosensors based on SWCNT-conducting polymer electrodes. *Analyst, 136*(7), 1279-1287.

Lenaz, G. (2001). The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. *IUBMB life, 52*(3-5), 159-164.

Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A.-G., . . . Stadtman, E. R. (1990). [49] Determination of carbonyl content in oxidatively modified proteins Methods in enzymology *186*, pp. 464-478): Elsevier.

Li, J. J. e., Muralikrishnan, S., Ng, C.-T., Yung, L.-Y. L., & Bay, B.-H. (2010). Nanoparticle-induced pulmonary toxicity. *Experimental biology and medicine, 235*(9), 1025-1033.

Marnett, L. J. (1987). Peroxyl free radicals: potential mediators of tumor initiation and promotion. *Carcinogenesis, 8*(10), 1365-1373.

Marshall, P. J., Warso, M. A., & Lands, W. E. (1985). Selective microdetermination of lipid hydroperoxides. *Analytical biochemistry, 145*(1), 192-199.

McCord, J. M., & Fridovich, I. (1969). Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *Journal of Biological chemistry, 244*(22), 6049-6055.

Mesquita, C. S., Oliveira, R., Bento, F., Geraldo, D., Rodrigues, J. V., & Marcos, J. C. (2014). Simplified 2, 4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. *Analytical biochemistry*, *458*, 69-71.

Nel, A., Xia, T., Madler, L., & Li, N. (2006). Toxic potential of materials at the nanolevel. *science, 311*(5761), 622-627.

Park, E.-J., Choi, J., Park, Y.-K., & Park, K. (2008). Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells. *Toxicology, 245*(1-2), 90-100.

Risom, L., Møller, P., & Loft, S. (2005). Oxidative stress-induced DNA damage by particulate air pollution. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *592*(1-2), 119-137.

Sattler, K. D. (2010). Cell Oxidative Stress: Risk of Metal Nanoparticles Handbook of Nanophysics 275-292,

Schieber, M., & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current biology, 24*(10), R453-R462.

Shvedova, A. A., Kisin, E., Murray, A. R., Johnson, V. J., Gorelik, O., Arepalli, S., . . . Sussman, N. (2008). Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis. *American Journal of Physiology-Lung Cellular and Molecular Physiology, 295*(4), L552-L565.

Shvedova, A. A., Pietroiusti, A., Fadeel, B., & Kagan, V. E. (2012). Mechanisms of carbon nanotube-induced toxicity: focus on oxidative stress. *Toxicology and applied pharmacology, 261*(2), 121-133.

Sies, H. (1991). Oxidative stress: introduction. Oxidative stress: oxidants and antioxidants, 15-21.

Sioutas, C., Delfino, R. J., & Singh, M. (2005). Exposure assessment for atmospheric ultrafine particles (UFPs) and implications in epidemiologic research. *Environmental Health Perspectives, 113*(8), 947-955.

Stellaa, G. M. (2011). Carbon nanotubes and pleural damage: perspectives of nanosafety in the light of asbestos experience. *Biointerphases, 6*(2), P1-P17.

Thannickal, V. J., & Fanburg, B. L. (2000). Reactive oxygen species in cell signaling. *American Journal of Physiology-Lung Cellular and Molecular Physiology, 279*(6), L1005-L1028.

Trotti, R., Carratelli, M., & Barbieri, M. (2002). Performance and clinical application of a new, fast method for the detection of hydroperoxides in serum. *Panminerva Medica, 44*(1), 37-40.

Ubezio, P., & Civoli, F. (1994). Flow cytometric detection of hydrogen peroxide production induced by doxorubicin in cancer cells. *Free radical Biology and medicine, 16*(4), 509-516.

Valko, M., Rhodes, C., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals

and antioxidants in oxidative stress-induced cancer. *Chemico-biological interactions, 160*(1), 1-40.

Vallyathan, V., & Shi, X. (1997). The role of oxygen free radicals in occupational and environmental lung diseases. *Environmental Health Perspectives, 105*(suppl 1), 165-177.

Warheit, D. B., Laurence, B. R., Reed, K. L., Roach, D. H., Reynolds, G. A., & Webb, T. R. (2004). Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. *Toxicological sciences, 77*(1), 117-125.

Xia, T., Kovochich, M., Brant, J., Hotze, M., Sempf, J., Oberley, T., . . . Nel, A. E. (2006). Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. *Nano letters, 6*(8), 1794-1807.

Zhang, Z., Berg, A., Levanon, H., Fessenden, R. W., & Meisel, D. (2003). On the interactions of free radicals with gold nanoparticles. *Journal of the American Chemical Society, 125*(26), 7959-7963.